

1-3-1923

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## Recommended Citation

Just, E. E., "The Fertilization-Reaction in Echinarachnius Parma. VIII. Fertilization in Dilute sea-Water" (1923). *Faculty Reprints*. Paper 116.  
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## THE FERTILIZATION-REACTION IN *ECHINARACHNIUS PARMA*. VIII.

### FERTILIZATION IN DILUTE SEA-WATER.

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The writer has shown,<sup>2</sup> by means of experiments made in 1920 at the Marine Biological Laboratory, Woods Hole, Mass., that a given dilution of sea-water with tap water which leaves the uninseminated egg of *Echinarachnius parma* intact is rapidly injurious to the inseminated egg during its process of membrane separation. After membrane separation the egg is likewise resistant to the action of the dilute sea-water. Certain experiments were also made during the same season to learn if it is possible to procure fertilization and development in diluted sea-water to which the inseminated egg during membrane separation is susceptible. These experiments were repeated during May and June of the 1921 season at the laboratory with essentially the same results.

In experiments made to discover if in a given dilution of sea-water, which is injurious to the inseminated egg of *Echinarachnius* during the period of membrane separation, it is possible to fertilize the egg, it was soon apparent that cleavage and normal gastrulation are not possible in a dilution which permits membrane separation. Thus during the period June 21 to June 28, 1921, inclusive, eggs from twenty-four females were inseminated in normal sea-water and in sea-water of varying dilutions. In all cases due precautions were taken as to the bulk of eggs, quantity of solution, and density of the sperm suspensions used in order that as far as possible conditions be made uniform. The results obtained with 95 per cent. sea-water (95 parts sea-water plus 5 parts tap water) and the dilutions ranging from this to 80 per cent. sea-water (80 parts sea-water plus 20 parts tap water) may be

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<sup>2</sup> Am. Jour. Phys., 1922, 61, 516.

summarized briefly as follows: In 95 per cent. sea-water eggs fertilize normally as revealed by the per cent. of membranes formed. They cleave and gastrulate in this dilution. In most cases the eggs are scarcely to be distinguished from eggs inseminated in normal sea-water. In 90 per cent. sea-water membranes are normal and cleavage fairly normally; but these eggs form about 10 per cent. exogastrulæ. In 85 per cent. sea-water the per cent. of membranes is close to normal—95 per cent. or slightly more. The cleavages show some abnormalities and the per cent. of exogastrulæ is greater than in 90 per cent. sea-water. In 80 per cent. sea-water the per cent. of cleavage falls and the per cent. of exogastrulæ increases.

The experiments indicated that the dilution made up with 75 parts sea-water plus 25 parts tap water is the lowest which permits cleavage. In this the gastrulæ are very abnormal. We may, therefore, turn our attention to the experiments made with this and greater dilutions of sea-water. Eggs inseminated in dilutions greater than those that permit cleavage separate membranes. Even in the dilution made up of equal parts tap water and sea-water some membranes separate, though these are often hard to see. Eggs, therefore, will respond to insemination with at least abortive cortical changes, though they can not cleave. I cite now one experiment which was made—as the majority of these experiments—on the eggs of three females.

June 21, 3:30 P.M. Six series of dilute sea-water, each consisting of 6 members, as follows:

No.	1.	2.	3.	4.	5.	6.
Parts sea water.....	50	55	60	65	70	75
Parts tap-water.....	50	45	40	35	30	25

Equal portions of uninseminated eggs from each of 3 females (*A*, *B*, and *C*) put in each dilution of sea-water to make Series *IA*, *IB*, and *IC*, *IIA*, *IIB*, and *IIC*.

Series *IA*, *IB*, and *IC* uninseminated.

Series *IIA*, *IIB*, and *IIC* inseminated with same quantity of sperm from one male. Inseminations in each series 5–25 seconds after eggs in dilute sea-water.

Controls: uninseminated eggs in normal sea-water; inseminated eggs in normal sea-water.

The eggs were examined at intervals. The next day, 7:50 A.M., the uninseminated eggs (Series IA, IB, and IC) showed an average of less than 10 per cent. cytolysis. The history of Series II. is given in the table below (Table I.):

TABLE I.

PER CENT. OF MEMBRANES AND OF CLEAVAGE IN EGGS OF *Echinarachnius* IN-  
IN SEA-WATER OF VARYING DILUTION.

No.	Amount of Sea-water in 100 Parts of Sea-water plus Tap Water.	Per Cent. of Membranes in Eggs from the 3 Females.			Per Cent. of Cytolysis Two Hours after Insemination.			Per Cent. of Cleavage 5 Hours after Insemination.		
		A.	B.	C.	A.	B.	C.	A.	B.	C.
1.....	50	0	2	1	18	4	9	0	0	0
2.....	55	0	2	0	4	2	1	0	0	0
3.....	60	7	7	7	0	3	0	0	0	0
4.....	65	5	22	20	0	1	0	0	0	0
5.....	70	14	63	47	0	3	0	0	0	0
6.....	75	60	95	87	0	2	0	5	16	4
Uninseminated control.....	100	0	0	0	0	0.1	0	0	0	0
Inseminated control.....	100	100	98	100	0	0.5	0	100	97	100

This experiment indicates that eggs inseminated in dilutions of sea-water may separate membranes, though they do not cleave. It would, therefore, be erroneous to assert—if membrane separation be the criterion for fertilization—that eggs that do not cleave have not been fertilized. Rather the failure to cleave is due to the action of the dilute sea-water in interfering with the cleavage mechanism—particularly with the activity of the hyaline plasma layer. There is no question here of “partial fertilization”; it is wholly a question of incomplete cleavage. This is important for the experiments that we may now consider.

In the experiments now to be considered it was repeatedly found that eggs may be inseminated in a dilution that is destructive to the egg which is inseminated in sea-water and exposed to this dilution during membrane separation. The criterion of this destructive action is the differential cytolysis of uninseminated and inseminated eggs before, during, and after membrane separation.

With cleavage, however, the case is quite different. That is, the per cent. of cleavage of eggs inseminated in a given dilution is no higher than that of eggs inseminated in sea-water and transferred to the dilution before, during, or after membrane separation. There might be a slight indication that eggs exposed during membrane separation show a lower per cent. of cleavage. I believe, however, that my figures on this point are not decisive.

I give now one experiment to show the per cent. of cytolysis in eggs inseminated in dilutions of sea-water as compared with that of eggs inseminated in sea-water and placed in the dilutions before, during, and after membrane separation.

June 27, 11:25 A.M. Following dilutions prepared:

No.	1.	2.	3.	4.	5.	6.
Parts tap-water.....	50	45	40	35	30	25
Parts sea-water.....	50	55	60	65	70	75

Five dishes for each of the six dilutions containing 10 c.c. each. Thus 5 series of 6 numbers each.

Drops of uninseminated eggs from one female added to dilutions as follows:

Series 1: 1 drop of eggs in each dilution.

Series 2: 1 drop of eggs in each dilution; inseminated immediately.

Series 3: Drop of eggs to each dilution 15 seconds after insemination in sea-water.

Series 4: Drop of eggs to each dilution during the period of membrane separation 35 seconds after insemination in sea-water.

Series 5: Drop of eggs to each dilution two minutes after insemination in sea-water.

Controls: Uninseminated eggs in normal sea-water; inseminated eggs in normal sea-water.

The results of this experiment follow:

12 M.

Series 1: Less than 1 per cent. cytolysis.

Series 2: As in Series 1.

Series 3:  
(12:00 M.)

No.	Dilution (Parts of Sea-water in 100 Parts of Solution.	Per Cent. of Eggs Intact.	Per Cent. of Eggs Cytolyzed.
1	50	79	21
2	55	77	23
3	60	89	11
4	65	98	2
5	70	97	3
6	75	100	0

Series 4:  
(1:00 P.M.)

1	50	50	50
2	55	47	43
3	60	85	15
4	65	80	20
5	70	90	10
6	75	97	3

Series 5:  
(1:30 P.M.)

1	50	69	31
2	55	83	17
3	60	86	14
4	65	97	3
5	70	99	1
6	75	98	2

Controls: Uninseminated in sea-water—1 per cent. cytolysis.  
Inseminated in sea-water 99+ cleavage.

It is clear from this experiment that eggs exposed to a dilution of sea-water during membrane separation cytolyze in slightly higher per cent. than eggs inseminated in the dilution. Similarly, eggs inseminated in normal sea-water and exposed before or after membrane separation cytolyze in slightly lower per cent. than eggs exposed during membrane separation. The cleavage per cent. of *surviving* eggs is about the same.

If we hold that membrane separation is a criterion of fertilization, then eggs inseminated in a given dilution of sea-water are fertilized whether they cleave or not. This admittedly is not a strong case, but it gives some support to the position that the fertilization reaction is practically instantaneous. It is not fertilization that is checked by the action of dilute sea-water, but the reactions which set up by fertilization lead to cell division.